

Myasthenia gravis: an update for the clinician

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J. P. Sieb

Department of Neurology, HELIOS

Hanseklinikum Stralsund, University Hospital

Bonn, Germany

Summary

This paper provides a thorough overview of the current advances in diagnosis and therapy of myasthenia gravis (MG). Nowadays the term ‘myasthenia gravis’ includes heterogeneous autoimmune diseases, with a postsynaptic defect of neuromuscular transmission as the common feature. Myasthenia gravis should be classified according to the antibody specificity [acetylcholine, muscle-specific receptor tyrosine kinase (MuSK), low-density lipoprotein receptor-related protein 4 (LRP4), seronegative], thymus histology (thymitis, thymoma, atrophy), age at onset (in children; aged less than or more than 50 years) and type of course (ocular or generalized). With optimal treatment, the prognosis is good in terms of daily functions, quality of life and survival. Symptomatic treatment with acetylcholine esterase inhibition is usually combined with immunosuppression. Azathioprine still remains the first choice for long-term immunosuppressive therapy. Alternative immunosuppressive options to azathioprine include cyclosporin, cyclophosphamide, methotrexate, mycophenolate mofetil and tacrolimus. Rituximab is a promising new drug for severe generalized MG. Emerging therapy options include belimumab, eculizumab and the granulocyte–macrophage colony-stimulating factor. One pilot study on etanercept has given disappointing results. For decades, thymectomy has been performed in younger adults to improve non-paraneoplastic MG. However, controlled prospective studies on the suspected benefit of this surgical procedure are still lacking. In acute exacerbations, including myasthenic crisis, intravenous immunoglobulin, plasmapheresis and immunoadsorption are similarly effective.

Keywords: diagnostics, myasthenia gravis, neuroimmunology, therapy/immunotherapy

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Correspondence: J. P. Sieb, Department of Neurology, HELIOS Hanseklinikum Stralsund, Grosse Parower Strasse 47–53, Stralsund 18435, Germany.

E-mail: j.sieb@klinikum-hst.de

Introduction

Disorders of neuromuscular transmission can be of immunological, toxic or genetic origin. Among these rare disorders, myasthenia gravis is the most frequent. The clinical hallmark of myasthenia (MG) gravis is a fluctuating pronounced weakness limited to the voluntary muscles. Char-

acteristically, muscular exertion increases the myasthenic weakness. It is a generalized disorder that often manifests initially as focal weakness. Eye muscle weakness at the onset of MG is evident in the vast majority of patients resulting in diplopia and ptosis. If the weakness is limited to the ocular muscles, it is designated ‘ocular myasthenia’. Oropharyngeal weakness may cause difficulties in

articulation, chewing and swallowing. In generalized myasthenia gravis, limb girdle weakness is typically more pronounced in the proximal than in the distal muscle groups. Myasthenic crisis is the life-threatening exacerbation of MG due to weakness of respiratory muscles and swallowing difficulties.

Surprisingly, epidemiological studies from Canada, Italy and Japan have observed an increasing frequency of MG in the elderly during recent decades [1–3]. In British Columbia, the annual number of first-time anti-acetylcholine receptor (AChR)-positive MG cases increased from 21.4/year/million during 1984–88 to 52.9 during 2004–08 in the elderly with an age of at least 65 years [1]. This international phenomenon might be a result of an increased awareness among medical doctors considering more frequently the diagnostic possibility of MG in the elderly.

Nowadays the term 'myasthenia gravis' describes a heterogeneous group of autoimmune diseases with a postsynaptic defect of neuromuscular transmission. These myasthenic syndromes can be divided according to the following categories with distinct clinical features and specific therapeutic needs:

- (1) course type:
 - ocular (in approximately 20% of MG patients)
 - oropharyngeal or generalized
- (2) age of onset:
 - start before puberty
 - early onset before the age of 50 years
 - late onset after the age of 50 years [4]
- (3) antibody specificity:
 - anti-AChR
 - anti-muscle-specific receptor tyrosine kinase (MuSK)
 - anti-low-density lipoprotein receptor-related protein 4 (LRP4)
 - seronegative MG
- (4) pathology of the thymus
 - normal/atrophic thymus pathology
 - thymitis
 - paraneoplastic occurrence associated with thymoma

In about 50% of those patients with ocular MG and in at least 10–15% with a generalized disease, the testing for autoantibodies to the AChR gives negative results. Some of these 'seronegative' patients have low-affinity antibodies to AChR that cannot be detected in standard solution phase assays, but can be detected in a novel method developed by a British group [5]. To increase sensitivity, recombinant AChR subunits were expressed with the clustering protein, rapsyn, in human embryonic kidney cells. Antibody binding to the AChR clustered at cell surface is visualized by immunofluorescence. Using this method, the British authors detected AChR antibodies to rapsyn-clustered AChR in two-thirds of sera previously negative for binding to AChR in solution. Unfortunately, this laboratory method for

detection of low-affinity anti-AChR antibodies has not been introduced commercially, and therefore it is not generally available for diagnostic purposes.

However, the seronegativity present in some MG patients does not result from the insufficient sensitivity of the applied laboratory method. In this subset of seronegative patients, myasthenic weakness comes from autoimmune processes directed to postsynaptic targets distinct from the AChR. Antibodies against MuSK are present in approximately 40% of seronegative cases with generalized MG, and in another portion of myasthenic patients, antibodies against LRP4 are detectable. MuSK and LRP4 are not involved directly in the neuromuscular transmission, but in the end-plate maturation. The membrane protein LRP4 is the receptor of glycoprotein agrin, which is released by the nerve terminal. Binding of agrin to LRP4 stimulates myotubes to form clusters of AChRs linked via rapsyn [6]. The MuSK is involved in this cellular signal processing. The anti-MuSK antibody-positive MG was identified in 2001 [7]. Little is still known about the MG with anti-LRP4 antibodies. This subtype was described independently in 2011 by two groups from Japan and Germany, respectively, [8,9]. The anti-LRP4 MG occurs predominately in middle-aged women. Overall, the clinical phenotype resembles closely that of anti-AChR-positive MG. It should be emphasized that even after the identification of anti-LRP 4 some MG patients are 'triple seronegative'.

Anti-MuSK antibody-positive MG

There are several clinically important differences between this type of MG and the anti-AChR antibody-positive type: only rarely is muscular weakness limited to the eye muscles, which are affected predominately in the majority of patients with anti-AChR antibody-positive MG. However, it is not possible to differentiate both types based only on clinical presentation. Anti-MuSK-positive MG occurs predominately in middle-aged women. Patients with anti-MuSK antibodies may have atypical presentations characterized by prominent oropharyngeal, facial, neck and respiratory muscle weakness. Facial and tongue atrophy may be present by clinical examination and by magnetic resonance imaging (MRI) [10]. The risk of myasthenic crisis is particularly high, and the chances of achieving complete stable remission are significantly lower than in anti-AChR MG [11]. As in the anti-AChR antibody-positive MG, neonatal myasthenia occurs in some newborns of myasthenic mothers due to transfer of maternal antibodies [12]. Furthermore, this type of MG can be also induced by D-penicillamine medication [13].

Immunological findings suggest that anti-MuSK MG may be different in aetiological and pathological mechanisms from the anti-AChR-positive MG. In anti-AChR MG, antibodies of the immunoglobulin (Ig)G1 and IgG3 subclass modulate the AChR, cause complement activation and

attract lymphocytes, acting together to decrease levels of the AChR and AChR-associated proteins and to reduce postsynaptic folding. In patients with anti-MuSK antibodies, there is no evidence of loss of junctional folds and no apparent loss of AChR density. Anti-MuSK antibodies are predominantly of the IgG4 isotype, which differs functionally from other IgG subclasses in its anti-inflammatory activity. The IgG4 antibodies do not cause complement activation at the end-plate. Moreover, IgG4 undergoes a post-translational modification termed 'Fab arm exchange' that prevents cross-linking of antigens [14]. The transfer of immunoglobulins from anti-MuSK-positive MG patients results in end-plate destruction in the recipient animals [15]. The anti-MuSK antibodies probably interfere with the cellular agrin-MuSK signalling cascade that leads to the formation of the AChR-rapsyn clusters. MuSK also anchors the collagenic tail subunit of acetylcholinesterase, and it was shown recently that passive transfer of anti-MuSK to mice reduces the size and density of ColQ to approximately 10% of controls [16].

For diagnosis, it is important to notice that needle electromyography (EMG) may show findings suggestive for myopathy in combination with abnormal spontaneous activity. This can easily be misinterpreted as evidence for inflammatory muscle disease [17]. Single-fibre EMG recordings of limb muscles give often negative results as these muscles are frequently spared and, on account of cholinergic hypersensitivity, many patients do not improve (or even do worse) with cholinesterase inhibitors. Therefore, pharmacological testing with intravenous administration of acetylcholinesterase inhibitors (e.g. edrophonium chloride, the so-called 'Tensilon test') is not useful in cases of suspected anti-MuSK MG [18]. As in the anti-AChR MG, there is a relationship between the individual anti-MuSK serum concentration and disease activity [19]. In MuSK-MG, thymus is normal or only very slightly affected [20,21]. It seldom shows germinal centres, and those that are present are small and similar to the small germinal centres that can be found in healthy thymus. There is also little or no activation of complement in the thymus. Thus, these observations suggest that the thymus in anti-MuSK myasthenia does not show any of the changes found in anti-AChR myasthenia patients. Considering the lack of evidence, we do not recommend thymectomy to our anti-MuSK MG patients.

Acetylcholinesterase inhibitors, such as pyridostigmine, are often poorly tolerated due to annoying muscle cramps. Intensive immunosuppression is often required. Administration of the anti-CD20 antibody rituximab (see below) often results in a long-lasting, outstandingly good therapeutic effect [22]. However, controlled prospective study-collected data on the use of rituximab in MG are not yet available. Apheresis and intravenous administration of immunoglobulins are used to overcome severe exacerbations of anti-MuSK MG [23].

Comorbidities

After confirmation of the diagnosis it is essential to identify comorbidities associated frequently with MG. Due to the possibility of a paraneoplastic aetiology, the search for a thymoma is mandatory. Thymoma-associated MG is generally associated with anti-AChR antibodies and not with anti-MuSK antibodies. In adults younger than 40 years of age, the presence of antibodies against titin or against the recombinant 30-kd titin fragment MGT30, respectively, reliably indicates a thymoma [24]. Titin is a large protein of the myofibrillar cytoskeleton. It is involved in the maintenance of the sarcomere and binds to the contact point between the A and I bands. Considering the low sensitivity and specificity of serological testing, computerized tomography (CT) or MR imaging studies are mandatory to rule out the presence of a thymoma. The treatment of patients with thymoma does not differ from those patients without thymoma but is complicated by the necessity to treat the neoplasm. Radiotherapy may be useful as adjuvant therapy in cases with incomplete surgical resection with microscopic or macroscopic residual thymoma tissue. Chemotherapy is considered a valid option in selected patients with residual disease after local treatments or to improve resectability of advanced tumours [25,26].

Furthermore, the following conditions are frequently observed in MG patients.

Association with other autoimmune diseases. Other autoimmune diseases are especially thyroiditis, rheumatoid arthritis or lupus erythematosus. Currently, the association of neuromyelitis optica spectrum disorder (NMOSD) with anti-aquaporin-4 antibodies and the anti-AChR antibody-positive MG has been described [27]. Both immune diseases occur at least 70 times more frequently in common than would be expected by chance. NMOSD develop almost exclusively in females with juvenile or early-onset MG [28]. MG frequently takes an unusually mild course in patients with NMOSD. A history of thymectomy could be a possible risk factor for the later development of NMOSD. NMOSD symptoms may begin with perennial latency after thymectomy [29]. In MG patients presenting with atypical motor or optic symptoms, testing for anti-aquaporin-4 antibodies should be ordered [28].

Inflammatory muscle disorders. In myasthenic patients, the risk of myocarditis is increased [30]. This association had already been described as 'Herzmyasthenie' by Leopold Laquer in 1901. The German term 'Herzmyasthenie' means, literally, myasthenia of the heart. Cardiac symptoms, such as shortness of breath and exercise intolerance, might be explained falsely by the existing MG. The possibility of associated myocarditis, especially during exacerbations of MG, must be kept in mind. Of more than 900 Japanese

patients, myocarditis was found in three and myositis in six individuals [31]. Myocarditis developed 13–211 months after the MG onset and was characterized by heart failure and arrhythmias. Myositis, developing before or at the same time as MG, affected limb and paraspinal muscles. Seven patients had one of these autoantibodies to titin, ryanodine receptor and muscular voltage-gated potassium channel Kv1.4, but not myositis-specific autoantibodies. Anti-Kv1.4 antibody-positive patients suffered from severe MG with bulbar symptoms, crisis, thymoma, myocarditis and prolonged QT time on electrocardiography. These results contrast with a study on 129 Caucasian patients [32]. In this cohort there were 22 (17%) anti-Kv1.4 antibody-positive patients, most of them women with late-onset MG.

Consequences of muscular weakness. Many patients complain of pain because of poor posture caused by the myasthenic weakness. Analgesic medication and orthopaedic therapy, including physiotherapy, may alleviate these frequent complaints. The risk of sleep apnoea syndrome is increased and there is a high prevalence of sleep disturbance among MG patients [33]. Sleep-related complaints may help to identify subjects at risk for abnormal breathing during sleep, even when daytime functional activity is judged normal.

Treatment options

Treatment of MG is based on four different options which take different amounts of time before muscular weakness will improve (Fig. 1):

- improvement of neuromuscular transmission by acetylcholine esterase inhibitors, e.g. pyridostigmine
- treatment of acute exacerbations (plasmapheresis, immunoadsorption, intravenous immunoglobulin)
- immunosuppression
- thymectomy

For each patient, an individual treatment plan must be compiled that will be adjusted further on the therapeutic response during the course. Using the spectrum of treatment options available nowadays, the majority of myasthenic patients can have largely normal lives. Even complete stable remission can be achieved in nearly every fourth patient suffering from anti-AChR or double-negative MG, respectively [11]. Unfortunately, treatment response is much less favourable in anti-MuSK MG, with a significantly increased risk of myasthenic crisis [34]. Experience shows, however, that therapeutic errors occur frequently in MG. For example, the hasty tapering of immunosuppressants is a common mistake resulting in even life-threatening exacerbations of the disease activity. Many myasthenic patients require life-long immunosuppressive therapy.

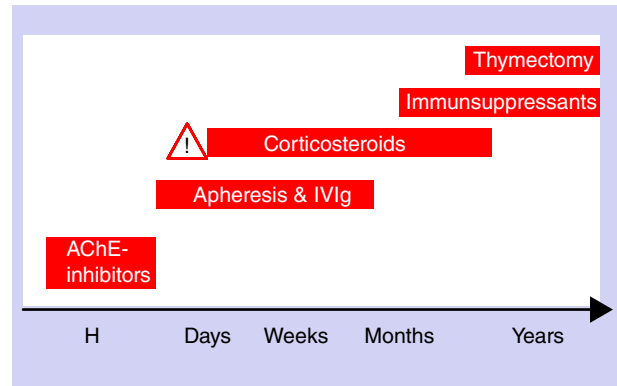


Fig. 1. Onset of action of the different therapy options in myasthenia gravis. The administration of acetylcholinesterase inhibitors (AChE) improves muscular weakness for several hours, but does not affect the course of the disease. At the beginning of corticosteroid therapy there is the risk of deterioration. Long-term therapy with corticosteroid should be avoided. With the use of immunosuppressive drugs, such as azathioprine, an effect on the myasthenic symptoms starts after several months of therapy. Plasma exchange or the intravenous immunoglobulins (IVIg) are used for myasthenic crisis. Both improve myasthenic weakness for just a few weeks. Thymectomy might influence beneficially the long-term course of non-paraneoplastic myasthenia gravis.

The current standard treatment of MG is hardly proven by controlled studies. It is largely based on serendipity and retrospective studies. For example, there are no studies on the potential benefit of an early and intensive immunosuppression similar to the ‘hit hard and early’ treatment concept in rheumatoid arthritis. Well-designed clinical trials comparing currently available therapeutic options are lacking. The choice of treatment modalities seems to rely mainly on institutional preferences and the personal experiences of the respective neurologist. However, conducting controlled trials on the therapy of rare and heterogeneous diseases, such as MG, is extremely difficult or even impossible. Moreover, poorly designed studies can even lead to fallacies.

With optimal treatment, the prognosis is good in terms of daily functions, quality of life and survival in the majority of patients suffering from MG. A thorough synopsis of the current standard therapy of MG is beyond the scope of this article. Symptomatic treatment with acetylcholine esterase inhibition is usually combined with immunosuppression. For decades, thymectomy has been performed in younger adults to improve non-paraneoplastic MG [35]. However, controlled prospective studies on the suspected benefit of this surgical procedure are still lacking. In view of the lack of controlled trials, it is not surprising that neurologists hold differing views to when, and even if, a thymectomy is indicated in the course of non-paraneoplastic MG. Probably the majority of neurologists recommend thymectomy to patients suffering from

generalized, early-onset MG and who are positive for anti-AChR antibodies. It is not recommended for anti-MuSK-positive MG (see above). Some neurologists believe that thymectomy is particularly beneficial if it is carried out early in the course of illness. It is unclear if thymectomy may reduce the risk of generalization in patients with purely ocular symptoms. A better understanding of the indications for surgery in non-paraneoplastic MG awaits well-designed prospective studies. In addition, there is no surgical consensus on whether the trans-sternal or the endoscopic procedure should be given preference [36,37]. The final data collection date for primary outcome measure of the MGTX trial will not be announced before August 2015 (ClinicalTrials.gov Identifier: NCT00294658). This international, prospective, randomized trial began in 2006 [38]. It aims to evaluate the impact of extended transsternal thymectomy on myasthenic symptoms, prednisone requirements and quality of life in patients with non-thymomatous, anti-AChR-positive MG.

Drugs improving neuromuscular transmission: acetylcholinesterase inhibitors

Pyridostigmine bromide is still the most commonly used acetylcholinesterase inhibitor in the treatment of MG, introduced more than 60 years ago [39]. The administration of acetylcholinesterase inhibitors is symptomatic therapy without affecting the course of the disease with the risk of intoxication at very high daily doses (so-called 'cholinergic crisis'). The daily dose of pyridostigmine bromide should exceed 600 mg only exceptionally. It is suitable as a long-term treatment in patients with very mild, non-progressive disease, and as an adjunctive therapy in patients with severe disease who are also on immunotherapy. In general, however, pyridostigmine provides only limited benefit in severely affected patients. Pyridostigmine may cause bradycardia, especially in elderly patients. Increased bronchial and oral secretions are a serious problem in patients with swallowing or respiratory insufficiency. Patients on higher daily doses frequently complain about excessive sweating, muscle cramps and diarrhoea. Switching medication to a sustained-release dosage form of pyridostigmine, which is available in only a limited number of countries (e.g. in the United States and Germany), may help to increase tolerability of this medication [40].

Anti-sense oligonucleotides present a novel type of AChE inhibition. AChE pre-mRNA is susceptible to alternative splicing. MG is associated with expression of the read-through transcript (AChE-R) which, unlike the normal 'synaptic' transcript (AChE-S), is not tethered to the post-synaptic membrane, but is a soluble monomer in the synaptic cleft. In experimental autoimmune MG (EAMG), inhibition of production of AChE-R using anti-sense oligonucleotides results in a significant reduction in synaptic expression of AChE-R. This improves muscle strength and

increases the survival rates of experimental animals with EAMG [41]. In humans there are only preliminary data on the therapeutic effect of Monarsen (EN101). This is a synthetic 20-base anti-sense oligodeoxynucleotide directed against the human AChE gene, modified to achieve stability for oral administration. Recent *in-vitro* and *in-vivo* studies indicate that EN101 is a Toll-like receptor (TLR)-9-specific ligand that can suppress proinflammatory functions and shift nuclear factor kappa B from the proinflammatory canonical pathway to the anti-inflammatory alternative pathway [42]. TLR-9 is a member of the TLR family, which plays a fundamental role in pathogen recognition and activation of innate immunity.

Treatment of acute exacerbations

Plasmapheresis, immunoadsorption and the intravenous administration of immunoglobulins, respectively, are used for crisis intervention. Only rarely do patients depend upon one of these therapies for a longer period of time [43]. Traditional plasma exchange entails removal of the pathogenic antibodies and other plasma components, such as soluble adhesion molecules and cytokines, separation from other blood components and then supplementation with 5% human albumin and crystalloids. The procedure may be carried out by plasma filtration techniques, plasma separation and more recently by antigen-specific immunoadsorption techniques that enable the return of non-pathogenic blood components to the patient. A standard course in MG entails five exchanges on alternating days utilizing 2–4 litres per exchange [44]. Venous access for plasma exchange can be achieved by central venous catheters or peripheral veins, and the preferred method varies among providers. Very recently, one retrospective study showed that peripheral veins access can be used successfully in most myasthenic patients and reduces the risk of serious and even lethal complications of the procedure [45].

A number of case reports and smaller, uncontrolled case series showed evidence for a roughly comparable clinical efficacy of plasmapheresis and immunoadsorption. However, the latter method avoids the necessity to substitute plasma replacement solution. This might result in better tolerability. Indeed, the first controlled study comparing the efficacy and safety of both treatments in myasthenic crisis confirms this advantage [46]. The use of high-dose intravenous immunoglobulin (IVIg) has gained wide application in the treatment of severe MG. Their mechanism of action is quite complex and not fully understood. IVIg seems to affect immune homeostasis by interfering at multiple levels, including modulation of the pathogenic autoantibody response, inhibition of complement activation and interference with the membrane attack complex formation, modulation of Fc receptors, down-regulation of the pathogenic cytokine responses and suppression of T cell function. The procedure usually entails the administration of 0.4 g/kg

body weight human pooled IgG over 3 or 5 days [44]. In acute exacerbations, including myasthenic crisis, intravenous Ig and plasma exchange have good and similar effects [47,48]. The major drawback of both is the relatively short-lived (in general up to 6 weeks) improvement in strength that makes the co-administration of longer acting immunosuppressive or immunomodulatory agents necessary.

Immunosuppressants

Azathioprine still remains the first choice for long-term immunosuppressive therapy. However, it is important to point out that there are only very limited data from controlled studies on the efficacy of azathioprine [49]. A significant disadvantage of azathioprine is the delayed onset of action. Commonly, azathioprine is therefore started combined with prednisolone to achieve a rapid therapeutic effect. Individually adjusted to the patient's needs, the prednisolone daily dose is then reduced gradually over a prolonged period of time. In a randomized double-blind study of 34 MG patients published in 1998, Palace *et al.* [49] compared prednisolone and azathioprine *versus* prednisolone alone who were followed-up for 3 years. One group received prednisolone (on alternate days) plus azathioprine (2.5 mg/kg); the other group received prednisolone on alternate days plus placebo. Initial high-dose prednisolone (1.5 mg/kg on alternate days) was tapered at remission to the minimal dose required to maintain remission. The prednisolone dose did not differ significantly between the two groups at 1 year but was reduced at 2 and 3 years in the azathioprine group. Patients with refractory disease or azathioprine intolerance are dependent upon alternative corticosteroid sparing immunosuppressive treatment options than azathioprine. Many patients with generalized MG require lifelong immunosuppression.

Corticosteroids are the least expensive, most reliable and rapid-acting drugs for immunomodulation in MG. This appraisal is based on general clinical experience rather than controlled studies. Their long-term use is complicated by severe and often intolerable adverse effects. The optimal manner to initiate corticosteroids depends upon the severity of weakness. It is important to note that with the initiation of higher doses, e.g. 60–100 mg prednisolone per day, some patients develop a significant exacerbation of myasthenic weakness, usually within the first day of treatment. The cause of this clinical phenomenon is elusive. In ocular MG a lower dose of prednisolone for a limited period of time, e.g. 20 mg for 2–4 weeks, can result in a pronounced improvement.

Cyclophosphamide. Cyclophosphamide pulses (500–1000 mg/m²) given every 4–12 weeks has been used occasionally for refractory MG. Alternatively, it can also be given orally in a daily dose of 1–2 mg/kg body weight. Cyclophosphamide may be used only cautiously because of its

myelotoxicity. Therefore, it is mandatory to evaluate again the necessity of the cyclophosphamide treatment after 6 months of treatment by omitting the medication or by tapering the daily dose, respectively. In pulse treatment, sufficient diuresis and the adjuvant mesna are required to reduce the risk of haemorrhagic cystitis. Additionally, myocardial damage, pulmonary fibrosis and cancer induction are possible consequences of the use of cyclophosphamide. Drachman *et al.* [50] treated three myasthenic patients, for whom treatment with thymectomy, plasmapheresis and conventional immunosuppressive treatment failed, using high-dose cyclophosphamide (50 mg/kg/day intravenously for 4 days) followed by granulocyte colony-stimulating factor. It is known that such immunoablative treatment with high-dose cyclophosphamide does not damage haematopoietic stem cells, permitting repopulation of the immune system without bone marrow transplant. All three patients showed marked improvement in myasthenic weakness.

Methotrexate

Methotrexate is a commonly used alternative to azathioprine. It is an anti-metabolite which has been used for decades in cancer therapy. In low doses, methotrexate is a generally safe and well-tolerated drug in the treatment of certain autoimmune diseases. However, there is only limited evidence for the effectiveness in MG. A recently published, single-blind study from South Africa provides evidence that methotrexate is an effective steroid-sparing agent 10 months after treatment initiation in generalized MG [51]. This study suggests that methotrexate has similar efficacy and tolerability to azathioprine.

Mycophenolate mofetil. Several retrospective case-series have suggested a very beneficial effect of this immunosuppressant. It is a reversible inhibitor of inosine monophosphate dehydrogenase in purine biosynthesis, which is necessary for T cells and B cells. Other cells are able to recover purines via a separate scavenger pathway and are thus able to escape the effect. Most patients well tolerate daily doses of 1–2.5 g. Two pivotal studies did not confirm the efficacy of mycophenolate in MG [52,53]. However, it is important to note the relevant limitations of both studies. For example, the observation periods of 12 and 36 weeks, respectively, appear to be too short for an assessment of the drug effect in MG. In the open follow-up, patients on mycophenolate monotherapy began to improve between 6 and 12 months. In the combination therapy group, prednisone dose decreased after 12 months [54]. After more than 24 months, 53% were off prednisone and 75% took less than 7.5 mg prednisone per day. This follow-up corroborates the experience of previous retrospective and pilot studies in demonstrating that mycophenolate is an effective treatment for myasthenic patients as either monotherapy or adjunctive therapy to prednisone. The long follow-up

demonstrated a steroid-sparing effect of mycophenolate during the second and third year of therapy that could not be demonstrated by studies of shorter duration. This illustrates that retrospective studies using rigorous outcomes measures can provide valuable information that may not be available from in short-term randomized controlled trials [54].

Cyclosporin and tacrolimus. Both are powerful immunosuppressive drugs used widely to prevent organ rejection after transplantation and in the treatment of autoimmune diseases. Although not structurally related to cyclosporin, tacrolimus has a similar mechanism of action and it has increasingly replaced cyclosporin for chronic immunosuppression after transplantation. Cyclosporin is a cyclic nonribosomal peptide of 11 amino acids and contains a single D-amino acid. Instead, tacrolimus has a macrolide lactone structure. Both inhibit the transcriptional activation of lymphokine and other genes required for T cell proliferation. The first step in mediating immunosuppressive effects is the binding to their respective intracellular receptors, the immunophilin. The drug/immunophilin complex binds to and inhibits the calcium/calmodulin-dependent serine/threonine phosphatase calcineurin. Under normal circumstances, calcineurin is responsible for activating the transcription of interleukin 2. Possible risks of both drugs include nephrotoxicity, encephalopathy, hypertension and tremor.

Cyclosporin is the immunosuppressive agent that revolutionized organ transplantation in the early 1980s by doubling the 1-year survival rate of cadaveric allografts. However, there are only scattered studies on the use of cyclosporin in MG [55–58]. In one controlled prospective and placebo-controlled study, 39 patients with steroid-dependent generalized MG received cyclosporin (5 mg/kg per body weight) or placebo for 6 months [56]. At the end of the study, patients in the cyclosporin group had significantly greater improvement in strength and a reduction in antibody titre. Percentage reduction of steroid medication was greater in the cyclosporin group, although the difference was not statistically significant. Cumulative side effects, however, caused a third of the patients to discontinue the medication; 10% did so secondary to slowly progressive nephrotoxicity. From our point of view, the limited evidence does not justify cyclosporin as a first-line immunosuppressant in MG. It is a reserve drug whose use is restricted due to serious side effects, numerous drug interactions, relatively high therapy costs and the necessity of regular measurements of the cyclosporin blood concentrations.

Tacrolimus suppresses the induction of experimental autoimmune myasthenianin rats [59]. In several small case-series tacrolimus lowered steroid requirements and induced stable remissions in MG [60–63]. Recently, a double-blind, placebo-controlled, parallel group study focused on the

ability of tacrolimus to reduce the corticosteroid dose in patients with stable myasthenic symptoms on prednisolone at doses equivalent to 10–20 mg/day [64]. Patients received tacrolimus or placebo for 28 weeks and the corticosteroid dose was tapered with the procedures specified in the study protocol. Unfortunately, this short-duration trial provided a disappointing result regarding the primary efficacy endpoint: the two study groups did not differ in the mean daily steroid dose. Besides immunosuppression, tacrolimus might have the potential to increase muscle strength by enhancing this ryanodine receptor function [65]. However, it is doubtful if this effect is indeed of clinical significance.

Emerging therapy options

Rituximab. The use of monoclonal antibodies with an innovative mode of action is promising, and might change the treatment of MG significantly in the coming years. A particularly promising candidate is rituximab. This is a genetically engineered chimeric mouse/human monoclonal antibody representing a glycosylated immunoglobulin with human IgG1 constant regions and murine light- and heavy-chain variable region sequences [66–69]. The antibody is produced by mammalian (Chinese hamster ovary) cell suspension culture. It is approved for the treatment of some lymphoma types and of severe active rheumatoid arthritis in adults who have had an inadequate response or intolerance to other disease modifying anti-rheumatic drugs. Rituximab binds specifically to the transmembrane antigen, CD20, a non-glycosylated phosphoprotein, located on pre-B and mature B lymphocytes. CD20 is found on both normal and malignant B cells, but not on haematopoietic stem cells, pro-B cells, normal plasma cells or other normal tissue. Toxicity studies have shown no other effect than the expected pharmacological depletion of B cells in peripheral blood and in lymphoid tissue. Peripheral B cell counts decline below normal following completion of the first dose of rituximab. B cell repletion begins within 6 months of treatment returning to normal levels between 9 and 12 months after completion of therapy [66,67].

Rituximab provides promising expectations for the treatment of MG, although no randomized controlled trials have been conducted so far. Case reports, retrospective small series and uncontrolled studies describe a long-lasting and pronounced clinical benefit due to rituximab even in severely affected, drug-resistant patients with MG [22,70–78]. Most interestingly, this might give better clinical benefit and last longer in anti-MuSK than in anti-AChR MG patients. Diaz-Manera *et al.* [22] treated six anti-MuSK- and 11 anti-AChR-positive MG patients with rituximab. All the patients included in this study were resistant to previous therapies and were classes III to V in the Myasthenia Gravis Foundation America classification. MuSK antibodies decreased dramatically during the follow-up after a single rituximab cycle. However, AChR antibodies remained at the

same titres during the same period of time. After a mean post-treatment period of 31 months, 10 of the anti-AChR patients improved but six of them needed reinfusions. In contrast, all anti-MuSK patients achieved a remission or minimal manifestations status and no reinfusions were needed. Consequently, in the anti-MuSK group, prednisone doses were reduced significantly and concomitant immunosuppressants could be withdrawn.

Infusion reactions including fever, chills and shivering are the most common side effects of rituximab. Pretreatment with hydrocortisone and diphenhydramine ameliorates these reactions. Very rare cases of progressive multi-focal leucoencephalopathy (PML) have been reported during the use of rituximab in lymphoma patients. The majority of patients suffering from this life-threatening complication had received rituximab in combination with chemotherapy. Cases of fatal PML have been reported following off-label use of rituximab for the treatment of certain autoimmune diseases, including systemic lupus erythematosus (SLE), rheumatoid arthritis and vasculitis. These patients with autoimmune diseases had a history of prior or concurrent immunosuppressive therapy and were diagnosed with PML within 12 months of their last infusion of rituximab [79–82]. To date, there are no reports on the occurrence of PML in patients treated with rituximab for MG.

Bortezomib. This is a proteasome inhibitor approved for treating patients with multiple myeloma, a plasma cell malignancy. Recent preclinical studies in cell cultures and animal models, and clinical studies in organ-transplant recipients, have demonstrated that bortezomib can kill non-neoplastic plasma cells within hours. This suggests that proteasome inhibitors could also be used for rapidly reducing autoantibody production in MG [83].

Belimumab. B cell activating factor (BAFF) is a potent B cell survival factor, and plays an essential role in B cell homeostasis and B cell function in the periphery [84,85]. Because BAFF is a crucial and potent factor for the survival and growth of B cells, both normal and autoreactive B cells compete for available BAFF. BAFF levels appear to regulate the survival threshold for B cells. Autoreactive B cells are poorly competitive for survival and they appear to be more dependent upon BAFF for their survival. BAFF levels are increased in the circulation of MG patients [86]. BAFF antagonists such as the monoclonal antibody belimumab may well provide new treatment options for MG. Belimumab is already approved in the United States, Canada and Europe for treatment of systemic lupus erythematosus.

Eculizumab. Complement activation at the neuromuscular junction may be the primary cause of acetylcholine receptor loss and failure of neuromuscular transmission seen in MG.

Eculizumab, a humanized monoclonal antibody, blocks the formation of terminal complement complex by selectively preventing the enzymatic cleavage of C5. It is the first therapy approved for the treatment of paroxysmal nocturnal haemoglobinuria. The results of one pilot Phase II study on the use of eculizumab in severe and refractory generalized MG are published in abstract form [87]. The purpose of this study was to determine whether or not eculizumab is safe and effective, despite treatment with various immunosuppressants that are currently available. The study has shown a significant clinical benefit of eculizumab in improving MG compared to placebo. Eculizumab was safe and well tolerated in all treated patients.

Etanercept. Etanercept blocks tumour necrosis factor (TNF)- α activity. It has European approval to treat rheumatoid arthritis, juvenile rheumatoid arthritis and psoriatic arthritis, plaque psoriasis and ankylosing spondylitis. In animals, it suppresses ongoing experimental MG [88]. This observation resulted in a small prospective trial on the effect in corticosteroid-dependent MG. Eleven patients were enrolled, with eight completing this 6-month trial. Two patients were withdrawn owing to disease worsening, and one patient was withdrawn because of an erythematous skin rash. Six of the eight patients who completed the trial improved, based on quantitative measures of muscle strength and lowering of corticosteroid requirement [89]. In addition to these disappointing study results, there are scattered case reports on patients who developed MG while taking etanercept and had resolution of symptoms after stopping it [90].

Granulocyte–macrophage colony-stimulating factor (GM-CSF). Forkhead box protein 3 (FoxP3) is a transcription factor necessary for the function of regulatory T cells (T_{regs}) [91]. T_{regs} maintain immune homeostasis and self-tolerance and play an important role in the prevention of autoimmune disease. *In-vitro* administration of GM-CSF enhances the suppressive function of T_{regs} and up-regulated FoxP3 expression in T_{regs} . There is one single case report on a patient with a prolonged myasthenic crisis refractory to conventional immunomodulatory treatments who was treated with GM-CSF [92]. This 77-year-old man received 750 μg GM-CSF daily for 3 days followed by 250 $\mu\text{g}/\text{day}$ for 3 days. After the fifth dose of GM-CSF, he had improved generalized strength and was eventually weaned from the ventilator.

The pillars of current MG therapy were already introduced more than 40 years ago. These are still pyridostigmine, corticosteroids, azathioprine and thymectomy. However, first experiences with new drugs, e.g. rituximab, are highly promising. It could be that the treatment of MG will change substantially until the end of this decade, but we are still far from a targeted immunotherapy.

Disclosure

The author discloses no conflicts of interest.

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